Göteborg, 2021

The Role of FCGBP in Mucus

Structure, Processing and Funtion

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Arvid Carlsson, Medicinaregatan 3, den 12 november, klockan 13.00.

av Erik Ehrencrona

Fakultetsopponent:

David J Thornton och Professor of Biochemistry

University of Manchester, UK

Avhandlingen baseras på följande delarbeten

I. **Ehrencrona, E**. van der Post, S. Gallego, P. Recktenwald, C. Rodriguez-Pineiro, A.M. Garcia-Bonete, MJ. Trillo-Muyo, S. Bäckström, M. Hansson, GC. Johanson, MEV. **The IgG Fc-binding protein FCGBP is secreted with all GDPH sequences cleaved, but maintained by inter-fragment disulfide Bonds**. Journal of Biochemistry **2021;** 293(1):100871.

II. Fakih, D. **Ehrencrona, E**. Martinez-Abad, B. Arike, L., Ermund, A. Trillo-Muyo, S. Gallego, P. Johansson, M.E.V. and Hansson, G.C. **The FCGBP Protein Induced at Lung Disease Anchors the Mucus Layer to the Tracheobronchial Surface.** Manuscript.

III. **Ehrencrona, E***. Gallego, P*. Garcia-Bonete, M.J. Trillo-Muyo, S. van der Post, S.V.P. Recktenwald, C.V., Rodriguez-pineiro, A.M. Hansson, G.C. and Johansson, M.E.V. **The FCGBP Structure Reveals a Convoluted C-terminal Dimer Stabilised by Cysteine Bonds.** Manuscript. *Equal contribution

IV. **Ehrencrona**, E. Svensson, F. Gallego, P. Garcia-Bonete, M.J. Martinez Abad, B. Hansson, G.C. Johansson, M.E.V. **Functional Analyses of FCGBP and its Role in Organisation of the Colonic Mucus Barrier.** Manuscript.

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

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Abstract

In this thesis, a bottom-up approach was used to study the IgG Fc-binding protein (FCGBP), which next to the MUC2 mucin is the second main component of secreted mucus in small intestine and colon. FCGBP is also found in airways during inflammatory conditions with static mucus. Although discovered as an IgG sequesterer, this function was not reproducible here using purified proteins. FCGBP includes many von Willebrand D domains (vWDs) with most having a GDPH (Gly-Asp-Pro-His) motif. Theoretically, hydrolysis of the DP peptide bonds should result in reactive Asp-anhydrides driving covalent crosslinking between FCGBP and MUC2. Using mass spectrometry (MS), recombinant proteins, electrophoresis and Western blot, we found that all motifs were cleaved but FCGBP remained intact as consecutive fragments were tethered by single disulphide bonds. Label-free MS quantification of proteins in murine mucus showed that Muc2 and Fcgbp are mostly not covalently bound, and *in silico* structural predictions further argued against such interactions, with these Asp-anhydrides being inaccessible for their suggested MUC2 substrates. Recombinant proteins were purified, analysed and used for generation of FCGBP antisera. The murine Fcgbp is smaller but highly similar to the human orthologue, making it ideal for functional and structural studies. Microscopy was used to study live and fixed tissue from mouse colon and airways to investigate its physiological role. Recombinant proteins formed C-terminal cysteine dimers with cryogenic electron microscopy (cryo-EM) showing a spring feather-like quaternary structure. Even larger linear structures were detected in cryo-EM micrographs, and electrophoresis showed large complexes in mucus. Immunohistochemistry (IHC) also revealed elongated ultrastructures in healthy intestine and airways of a murine chronic obstructive pulmonary disease (COPD) model. Results indicated less attached mucus in airways of *Fcgbp*$^{-/-}$ mice, and further a mucus expansion phenotype in colon. An N-terminal sequence linked to helical gliding was studied and alignments revealed that the murine sequence had partially been genetically lost. The remaining N-terminal sequence shared between human and mouse was found to be repeated prior to every vWD in the FCGBP sequence. In summary, these results indicate a role for FCGBP in mucus structure and attachment.

Keywords: Mucus, FCGBP, IgG, IBD, COPD, Mucins, Disulphide, GDPH

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